DATA EVALUATION RECORD

STUDY 7c

CHEM 112600 Prohexadione calcium §163-1
CAS No. 127277-53-6
FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 44457790
O'Connor, J. 1992. BX-112: BBA plant protection product evaluation: Determination of the seepage behavior of BX-112 by soil column studies (aged test). LSR Report No.: 91/1213. BASF Reg. Document No.: 92/11035. Unpublished study performed by Life Science Research Ltd., Suffolk, ENGLAND; and submitted by BASF Corporation, Agricultural Products, Research Triangle Park, NC.

DIRECT REVIEW TIME = 54 Hours

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CONCLUSIONS

Mobility - Leaching & Adsorption/Desorption

1. This study is not scientifically valid and not acceptable for the partial fulfilment of the data requirement for a soil mobility of prohexadione calcium degradates (column leaching). Cyclohexene ring-labeled [3,5-^{14}C]prohexadione calcium, applied at a nominal concentration of 0.1 mg/kg and aged for 13 hours in sand soil adjusted to 40% of the maximum soil water-holding capacity and incubated at 20 ± 1 °C in darkness, appeared to have a low mobility in sand soil (German Speyer 2.1) columns compared to atrazine. The columns were leached over a period of two days, with equivalent of ca. 8 inches distilled water. The test design and analytical method were inadequate to accurately determine the mobility of the test compound and its degradates.

2. This study does not meet Subdivision N Guidelines for the partial fulfillment of EPA data requirements on soil mobility (column leaching) for the following reasons:

   (i) the elution volume was not equivalent to 20 inches;

   (ii) the leaching solution was not 0.01-0.02 *N* $CaCl_2$ solution;

   (iii) the soil treatment rate was lower than the lowest proposed application rate;

   (iv) residues were not characterized after aging/prior to leaching;

   (v) following column leaching the material balance was below the acceptable range (90-110%); and

   (vi) only foreign soil was utilized in the study.

3. To satisfy the Subdivision N data requirement the Registrant should repeat the aged leaching study considering the above comments elaborated in The Reviewers Comments section of this review.

METHODOLOGY

Test Substance: Cyclohexene ring-labeled [3,5-^{14}C]prohexadione calcium, calcium 3-oxido-4-propionyl-5-oxo-3-cyclohexene-carboxylate; radiochemical purity 98.4%, specific activity 3.62 MBq/mg;

Reference Substance: [C^{14}]-atrazine, radiochemical purity 98.7%, specific activity 4.28 MBq/mg;

Soil Tested: Sand soil, 2 mm sieved and stored for no longer than 3 months, German Speyer 2.1 containing 87.4% sand, 9.1% silt, 3.5% clay, 0.7% organic carbon, pH 6.1%, CEC 4.9 meq/100 g (p. 15 see attachment).

Test Design: Duplicate samples of sand soil were placed in biometer flasks and adjusted to 40% of the maximum water-holding capacity. Samples were pre-incubated for 19 days at 20 ± 1.5 °C in darkness. The soil microbial biomass was estimated using the chloroform fumigation-incubation method. Results indicated that the soil was viable, the soil microbial biomass was 20.29 mg C/ 100 g soil.

Pre-incubated soil samples were treated with cyclohexene ring-labeled [3,5-^{14}C]prohexadione calcium, dissolved in distilled water, at a nominal rate of 0.1 mg/kg. The soil was aerobically aged in an incubator for 13 hours (approximate one-half life of a.i.) at 20 ± 1 °C in darkness. In the incubator, humid air was passed through the biometer flasks containing soil and connected to the volatile organics (2-ethoxyethanol) and carbon dioxide (mono-ethanolamine:water; 20:80, v:v) traps. Soil samples were not analyzed following the aging period.

Next, glass columns (5-cm internal diameter x 48 cm in length) equipped with conical bottoms (filled with siliceous sand) were packed (while agitating) to a depth of 28 cm with untreated, sieved (2 mm) sand soil (p. 21; diagram p. 87); columns were saturated with distilled water (p. 22). The aged treated soil was added on top of the soil columns as a layer of approximately 2 cm (as the 28- to 30-cm layer); duplicate columns and control columns were utilized. Two additional soil columns were treated with [^{14}C]atrazine for comparison. To the top of each column and to the leachate collection flasks CO_2 traps (1 M KOH and mono-ethanolamine:water; 20:80, v:v) were connected (Figure 1, p. 32). The columns were leached with 393 mL of distilled water over a period of 48 hours (p. 22; see Comment # 4) in darkness at room temperature. The leachate was collected in 100-mL fractions into the leachate collection flasks connected to the bottom of the columns.

Sample Analysis: Aliquots of the leachate fractions were analyzed for total radioactivity by LSC (p. 23). Leachate fractions were placed in sealed glass containers connected to two CO_2 (0.1 M sodium hydroxide) traps in sequence. Fractions were acidified with 10 *N* sulfuric acid, aerated for 2 hours, and treated with nonradiolabeled 3,5-dioxo-4-propionyl-cyclohexane-1-carboxylic acid (KI-2817). The fractions were extracted three times with chloroform; the method of extraction was unspecified. The organic and aqueous phases were brought to volume and duplicate aliquots were analyzed for total radioactivity by LSC.

The [^{14}C]-BX 112 fortified soil columns were divided into five 6-cm sections. Each section was placed in a sealed glass container connected to two CO_2 (0.1 M sodium hydroxide) traps in sequence. The soil samples were acidified with 1 *N* sulfuric acid and aerated for two hours. Samples were extracted by shaking with acetone, filtered, and the filtrate rinsed with acetone. The combined filtrates were concentrated under vacuum and treated with nonradiolabeled KI-2817. The filtrate was further extracted three times with chloroform; the method of extraction

was unspecified. The organic and aqueous phases were brought to volume and duplicate aliquots were analyzed for total radioactivity by LSC. Duplicate subsamples of the post-extracted soil were analyzed for total radioactivity by LSC following combustion (p. 24).

Each [^{14}C]-atrazine fortified soil was shaken with acetone and 1 *N* sulfuric acid, filtered, and filtrate was rinsed with acetone. Combined filtrate was concentrated to 40 ml and three times extracted with chloroform. Both organic and aqueous phases were brought up to known volumes and the radioactivity was determined via LSC. Extracted soil was combusted and analyzed for radioactivity.

Also, trap contents were analyzed for radioactivity. Leachate and soil extracts containing >0.001 ppm were analyzed by TLC to characterize [^{14}C]residues (p. 24; *see Comment #10*). Method details of the TLC analysis were not reported.

Sample Storage: Prior to analysis eluate samples were stored in a refrigerator at +4 °C, whereas soil samples were stored in a freezer at approximately -20 °C.

THE AUTHOR'S DATA SUMMARY

Based on column leaching studies, cyclohexene ring-labeled [3,5-^{14}C]prohexadione calcium (radiochemical purity 98.4%), applied at a nominal concentration of 0.1 mg/kg and aged (13 hours) in a sand soil adjusted to 40% of maximum soil water-holding capacity and incubated at 20 ± 1 °C in darkness, appeared to have a low mobility in the sand soil columns which were leached over a period of two days with distilled water. However, the Reviewer notes that mobility determinations were made for total residues (parent compound plus degradates), as individual data were not reported.

Table 1 presents the total radioactivity distribution in the column soil sections, leachate (i.e., aqueous and organic extract, extracted eluent fraction), and traps. Table 2 presents the percent of radioactivity recovered in each soil segment of the BX-112 and atrazine columns.

During aerobic incubation and the column elution test, about 61.2% of applied [^{14}C]-BX-112 was released as [^{14}C]-CO_2. Only 0.3% of applied [^{14}C]-atrazine was released as [^{14}C]-CO_2 (see Table 1). [C^{14}]-CO_2 released during BX-112 aerobic incubation in soil accounted for as much as 46.6% of radioactivity. The majority ofradioactivity recovered from the soil (i.e. 91.5%) was located in the top 12 cm of the BX-112 columns (see Table 2). The total radioactivity in the 24-to 30-cm depth (top layer, including the application layer) was 12.1% and 20.4% of the applied for the first and second column, respectively. In the 18-to 24-cm depth (second layer from top), 6.8% and 0.1% of the applied was present in the first and second column, respectively. Radioactivity present in the leachate (designated as wash) of the first column was only 1.6% of the applied. The radioactivity recovered from the [^{14}C]-atrazine treated column was detected throughout the column with the maximum located in the 12-18 cm segment (i.e. 34.8%) and the minimum at the

bottom of the column. The comparison of radioactivity distribution in the column fortified with [^{14}C]-atrazine and aged [^{14}C]-BX-112 treated soil showed that atrazine had greater mobility in soil than BX-112. Atrazine according to Helling (1917) has medium mobility, therefore BX-112 was characterized as having a low mobility in German Speyer 2.1 soil.

The material balance based on LSC analysis was 78.8-84.3% (avg. 81.6%) of the applied radioactivity for the two BX-112 columns (Table 1, p. 34). The study author explained the low recovery as due to BX-112 rapid aerobic degradation and losses of [^{14}C]-CO_2 during soil transfer after ageing, and column separation after elution. The data varied between replicate columns significantly. The material balance was 91.55-86.1% (avg. 88.8%) for atrazine columns.

THE REVIEWER'S COMMENTS

1. This study is not scientifically valid and not acceptable for the partial fulfilment of the data requirement for soil mobility of prohexadione calcium (column leaching). The leaching study indicated that the parent compound and its degradates were less mobile in sand soil (German Speyer 2.1) than atrazine which, according to Helling (1971), has medium mobility in Speyer 2.1. BX-112 and its degrades did not leach below 12 cm whereas atrazine leached down the column with a maximum concentration in a 12 to 18 cm column depth. The test design and analytical method were inadequate to accurately determine the mobility of the test compound and its degradates for the following reasons:

   A. The [^{14}C]residues in the aged soil were not characterized after aging/prior to leaching. The total radioactivity and the percentage of the applied present as parent could not be determined. It could not be determined whether a sufficient amount of parent compound remained for the determination of soil mobility (≥50% of the applied radioactivity) following the 13-hour aging period. The study author stated that the soils were aged for 13 hours, which was approximately one half-life of the test compound based on the results of one aerobic metabolism study (LSR Report No: 92/KCI118/0272, study not submitted).

   B. Following the leaching study mobility determinations were made for total residues (parent compound plus degradates), as individual data were not reported and the percentages present as parent or its degradates could not be determined. Data varied significantly between replicate columns.

   C. The soil columns were not sufficiently eluted. The elution volume was ca. 8 inches (393 mL) while it was supposed to be an equivalent of 20 inches which is 997 ml {i.e.; $((\pi^*$ (column diameter)$^2)/4)^*$ 20 inches = (3.1416*5*5)/4 *50.8=997 ml)}. Only a sufficient elution volume can provide downward movement of the parent and its degradates through the soil column.

D. The leaching solution was not 0.01-0.02 *N* $CaCl_2$ solution but distilled water. The use of distilled water could cause soil particles to disperse, decreasing the rate of infiltration and leaching. Additionally, the use of distilled water may lead to the removal of sorbed ions from soil particles, thereby affecting the degree of adsorption of the test material.

E. The soil treatment rate was not equal to the highest recommended rate for a single application. The fortification rate used in this study was equivalent to 0.1 ppm. Therefore, it was lower than the lowest proposed application rate. The lowest proposed application rate is 0.125 ppm lb a.i./acre, applied three times per season, for peanuts and the highest is 0.825 ppm lb a.i./acre, applied twice per season, for apples.

F. The bulk densities of the packed soil were not reported. Soil bulk densities should be measured and reported, and should be similar for all columns (hand packed) of the same soil.

G. The material balance was 81.6% thus below the acceptable 90-110% range . The study authors explained that the obtained recovery was due to instability of prohexadione calcium due to its rapid degradation, and perhaps loss of radioactive CO_2 during aged soil transfer to column for leaching test.

H. The soil moisture content was not adjusted to 75% of 0.33 bar prior to the pre-incubation period. The soil moisture was adjusted to approximately 40% of the maximum water-holding capacity (p. 20). The study author did not report the relationship between the two moisture contents for the test soil.

I. Only foreign soil was utilized in this study. The soil may be not representative of the soils in the typical peanuts/apple growing areas of the United States.

J. All data were reported adversely with respect to depth of leaching. The top of the column was designated as 30 cm, while the bottom was designated as 0 cm.

K. The study author stated that chromatographic analysis of organic extracts from the leachates and soil column extracts was not necessary, as the concentration of radiolabeled material in these samples was less than 0.001 ppm. The reviewers note that the concentrations were low because the study were not conducted in the highest recommended label rate but lower than the lowest application rate for the parent (peanuts: 0.125 lb a.i./acre three times per season; apples: 0.825 lb a.i./acre twice per season). HPLC or GC/MS analysis are always highly recommended.

L. The study author stated that the limit of detection was derived statistically from background counts (p. 18); however, the limits of detection and quantitation were not reported. The reviewers note that prohexadione calcium residues,

although in very low levels, were present throughout the column length. It is necessary that both limits of detection and quantitation be reported to allow the reviewer to evaluate the adequacy of the method.

REFERENCES

Helling. C.S., 1971. Pesticide mobility of soils II., Application of soil thin layer chromatography. *Soil Sci. Soc. Amer*, Proc., **35**, pp 737-743.

McCall P.J., Swann R.L., Laskowski D.A., Unger S.M., Vrona S. A. and Dishburger H.J., 1980 Estimation of Chemical Mobility in Soil from Liquid chromatographic Retention Times. Bull. *Environ. Contam. Toxicol.* **24**, pp190-195.

Prohexadione calcium

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